and 6 possible sites of N-linked glycosylation.[5] Recently, the sequences of the PLTP gene were identified and found to be similar to human cholesteryl ester transfer protein (CETP).[5,6] PLTP and CETP have the same number of amino acids and both share 20% homologous sequences.[5,6] In addition, both proteins assist the transfer of lipids from lipoproteins, suggesting that they are possibly derived from the same ancestor.[1]

Physiological functions of PLTP have not been clearly verified yet. Similar to that of CETP, it was reported that the activity of PLTP is regulated by lipids.[3,7] The transfer activity of cholesterol by plasma CETP was stimulated in rabbits after a high cholesterol and high fat diet via an increase in the levels of plasma CETP and CETP mRNA in the liver.[8,9] An in vitro study showed that palmitic acid inhibits HDL conversion by PLTP.[3] The addition of cholesterol also suppressed the activity of PLTP to transfer phosphatidylcholine.[3] Recently, an in vivo study observed that PLTP activity in plasma, phospholipids in HDL, and PLTP mRNA levels in the lung significantly increased in rats after a high fat and high cholesterol diet.[7] However, PLTP activity in plasma and PLTP mRNA levels in the liver, adipocytes, and lung decreased in rats after lipopolysaccharide injection.[7] A previous study showed that postprandial lipidemia enhances the activity of PLTP in transferring phospholipid from lipoprotein into HDL and in facilitating the size and composition conversion of HDL (Zoppo *et al.*, unpubl. data). PLTP expression and activity are affected by adding lipids. It is hypothesized that exogenous lipids may mediate PLTP expression in the liver, the major organ for PLTP synthesis, by plasma lipoproteins or insulin during the fed state. The purpose of the study was to investigate the effects of lipoproteins or insulin on PLTP expression in HepG2 cells.

MATERIALS AND METHODS

Cell Line and Reagents

Human hepatoblastoma cells (HepG2, ATCC HB-8065, CRCC 60025) were purchased from Culture Collection and Research Center (CRCC) at Food Industry Research and Development Institute (Hsinchu, Taiwan). Minimum essential medium (MEM) powder containing non-essential amino acids, fetal bovine serum (FBS), sodium bicarbonate, trypsin-EDTA, penicillin, streptomycin, sodium pyruvate, trypan blue, bovine insulin, protein molecular weight standards, bovine serum albumin (BSA), phosphate buffered saline (PBS), Coomassie Brilliant Blue R-250, stacking gel buffer, resolving gel buffer concentrate, 40% (w/v) acrylamide bisacrylamide (37.5:1), sodium dodecyl sulfate (SDS), agarose, formamide, and glycerol were obtained from Life Technologies GIBCO BRL (Taipei, Taiwan). HDL, LDL, and VLDL were purchased from Calbiochem-Novabiochem International (Taipei, Taiwan). Bicinchoninic acid (BCA) protein assay and RNA isolation kits were purchased from PIERCE (Taipei, Taiwan) and Gentra System (Taipei, Taiwan), respectively. Tris base, ammonium persulfate, and Illuminator™ chemiluminescent detection system, secondary antibody (anti-fluorescein antibody-alkaline phosphatase conjugate), and Prime-It Fluor fluorescence labeling kit were purchased from Stratagene (Taipei, Taiwan). Bromophenol blue and 37% formaldehyde were purchased from Tokyo Chemical Industry (Taipei, Taiwan), and Fisher Scientific International (Taipei, Taiwan), respectively. Morpholinopropane sulfate (MOPS) and β-mercaptoethanol were obtained from Sigma (Taipei, Taiwan). The Western-Light™ chemiluminescent detection system and secondary antibody (goat anti-mouse IgG-alkaline phosphatase conjugate) were provided by Tropix (Taipei, Taiwan).

Cell Culture and Treatments

HepG2 cells were grown in MEM, containing 10% FBS, 1 mM sodium pyruvate, 50 U/mL penicillin and 50 μg/mL streptomycin, at 37 °C in a humidified CO_2 incubator (95% air and 5% CO_2). Cells were collected by using 0.25% trypsin and 0.02% EDTA solution 8 to 10 days after original plating. Cell were then subcultured by seeding in 75 cm^2 flasks at a density of 1.0×10^6 and grown to approximately 90% confluency. Prior to treatments, cells were synchronized by incubating in basal medium (serum-free complete medium without antibiotics) for 24 h to eliminate interfering factors present in the serum. Cells were then incubated with HDL (50 μg protein/mL),[10] LDL (50 μg protein/mL),[11] VLDL (50 μg protein/mL), or insulin (1 μg protein/mL)[12] for another 12-24 h. The medium was not changed during the incubation period. The conditioned medium was collected for protein assay at 12